S1P-Mediated Endothelial Cell Migration into Porous Poly(ethylene glycol) Modular Scaffolds Formed in the Presence of HepG2 Cells

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Statement of Purpose: We have exploited the lower critical solution temperature (LCST) of poly(ethylene glycol) (PEG) in high salt solutions [1] to develop novel microspheres without the use of surfactants or organic solvents. The versatility of PEG derivatives allows the microspheres to be tailored for specific applications and bioactivity. Furthermore, different PEG microspheres can be crosslinked together to form modular scaffolds containing properties dependent on the types of microspheres used in their formation. These scaffolds can be crosslinked in the presence of cells using a variety of molecules including proteins in the cell culture medium or enzymatically-degradable peptides. Here, we present a scaffold designed for the long-term culture of hepatocytes (HepG2 cells) and with bioactivity intended to promote vascularization via endothelial cell (EC) infiltration. This was accomplished by combining three separate types of microspheres: 1) 100% PEG microspheres containing RGD peptides for structural support, biocompatibility, and cell adhesion, 2) 100% PEG microspheres that undergo hydrolysis under physiological conditions to provide porosity, 3) microspheres containing bovine serum albumin (BSA) for the delivery of sphingosine-1-phosphate (S1P), a bioactive lipid that enhances EC migration.

Methods: PEG-octa-vinyl sulfone (PEG-OVS, MW 10K) and PEG-octa-amine (PEG-OAm, MW 10K), PEG-OVS and BSA, or PEG-octa-acrylate (PEG-OAc, MW 10K) and PEG-OA were crosslinked in PBS, pH 7.4 at 37°C until d_{PCS} = 100 nm microgels formed. BSA solutions were preincubated with S1P (final concentration of 3 uM). Microspheres were formed by mixing each solution with the required volume of 1.5 M sodium sulfate in PBS to allow the LCST of the microgels to be reached at 37°C. PEG-OVS/PEG-OAm microspheres were formed in the presence of 0.2 mM RGD peptide (GCGYGRGDSPG). Microspheres were buffer exchanged into endothelial growth medium (EGM) containing 2% fetal bovine serum (FBS), combined into a single container with $1x10^5$ HepG2 cells labeled with a green fluorescent dye (Vybrant DiO), and centrifuged at 1000 RCF for 10 min. The scaffold was allowed to crosslink overnight at 37°C and then incubated with $1x10^5$ cells/mL of EC's labeled with a red fluorescent dye (Vybrant DiI). After incubation at various timepoints between 16 h and 48 h, cell migration within the scaffolds was assessed using confocal microscopy. Scaffolds without cells were also formed as described above using microspheres fabricated with FITC-labeled PEG-OAm.

Results: FBS proteins present in the EGM were able to crosslink PEG-OVS/OAm, PEG-OVS/BSA, and PEG-OAc/OAm microspheres into structurally stable scaffolds following compaction by centrifugation. PEG-OVS/BSA and PEG-OAc/OAm microspheres were found by confocal microscopy to be well-mixed and distributed throughout the scaffolds. PEG-OAc/OAm microspheres were completely hydrolyzed after a 12-h incubation at 37°C to provide a porous scaffold for EC infiltration. HepG2 cell viability was unaffected by the centrifugation used during scaffold formation as evidenced by fluorescein diacetate staining. Confocal microscopy revealed ECs to infiltrate the scaffolds at a rate dependent on the concentration of preloaded S1P, and no migration was observed in the absence of RGD peptide. ECs migrated at up to 4.5 um/h and were observed to be intermingled with the HepG2 cells (figure 1).

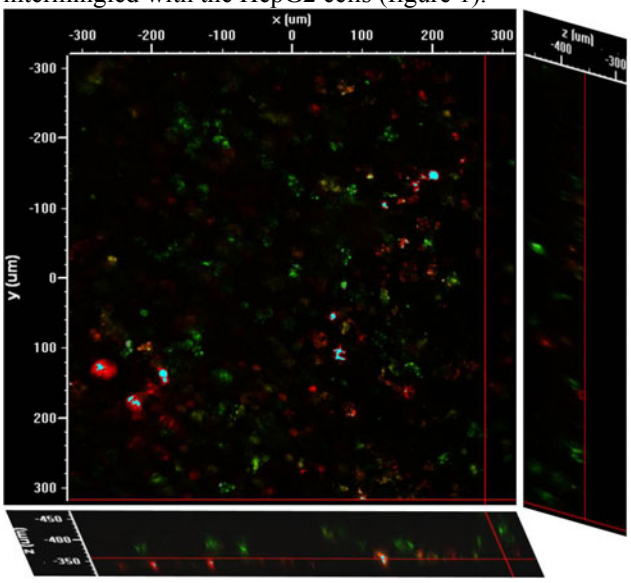

Figure 1. Confocal microscopy of labeled HepG2 (green) and endothelial (red) cells after a 17 h incubation within a porous microsphere scaffold preloaded with S1P. The z-axes display sections (red lines) of the scaffold perpendicular to the x/y plane. The z-axis perpendicular to the x-axis displays endothelial cells that have migrated 75 um into the scaffold.

Conclusions: We were able to fabricate modular scaffolds with porosity and bioactivity dependent on the properties of the microspheres contained within them. Seeding of cells into scaffolds was accomplished both by crosslinking microspheres in the presence of cells or by incorporating integrin motifs into the scaffolds to allow directed cellular infiltration. Incorporation of S1P-loaded microspheres demonstrated the ability to influence cell migration through the delivery of a chemotactic agent. By combining microspheres into modular scaffolds under physiological conditions, we have developed a simple, tunable method of culturing cells in a porous 3D environment.

References:

1. Bailey FE. J App Poly Sci. 1959;1:56-62.